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(FILE 'HOME' ENTERED AT 10:34:01 ON 10 FEB 2004)

FILE 'STNGUIDE' ENTERED AT 10:34:35 ON 10 FEB 2004

FILE 'HOME' ENTERED AT 10:34:38 ON 10 FEB 2004

INDEX 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, AQUASCI, BIOBUSINESS, BIOCOMMERCE, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA, CANCERLIT, CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DISSABS, DDFB, DDFU, DGENE, DRUGB, DRUGMONOG2, ...' ENTERED AT 10:34:50 ON 10 FEB 2004

| SEA | YEAST |
|---|---|
| 351 | FILE ADISCTI |
| 114 | FILE ADISINSIGHT |
| 83 | FILE ADISNEWS |
| 34077 | FILE AGRICOLA |
| 858 | FILE ANABSTR |
| 2366 | FILE AQUASCI |
| 9394 | FILE BIOBUSINESS |
| 2064 | FILE BIOCOMMERCE |
| 123036 | FILE BIOSIS |
| 38024 | FILE BIOTECHABS |
| 38024 | FILE BIOTECHDS |
| 51718 | FILE BIOTECHNO |
| 33468 | FILE CABA |
| 10059 | FILE CANCERLIT |
| 179473 | FILE CAPLUS |
| 9079 | FILE CEABA-VTB |
| 142 | FILE CEN |
| 1313 | FILE CIN |
| 3871 | FILE CONFSCI |
| 92 | FILE CROPB |
| 1409 | FILE CROPU |
| 8127 | FILE DISSABS |
| 2527 | FILE DDFB |
| 3980 | FILE DDFU |
| 62216 | FILE DGENE |
| 2527 | FILE DRUGB |
| 147 | FILE DRUGMONOG2 |
| 86 | FILE IMSDRUGNEWS |
| 5643 | FILE DRUGU |
| 106 | FILE IMSRESEARCH |
| 772 | FILE EMBAL |
| 71412 | FILE EMBASE |
| 40999 | FILE ESBIOBASE |
| 4398 | FILE FEDRIP |
| 248 | FILE FOMAD |
| 458 | FILE FOREGE |
| 13429 | FILE FROSTI |
| 26688 | FILE FSTA |
| 199909 | FILE GENBANK |
| 261 | FILE HEALSAFE |
| 10808 | FILE IFIPAT |
| 343 | FILE IMSPRODUCT |
| 15496 | FILE JICST-EPLUS |
| 257 | FILE KOSMET |
| 57116 | FILE LIFESCI |
| 112 | FILE MEDICONF |
| 85331 | FILE MEDLINE |
| 602 | FILE NIOSHTIC |

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             1718   FILE NTIS
               55   FILE NUTRACEUT
              512   FILE OCEAN
            52409   FILE PASCAL
              161   FILE PHAR
               66   FILE PHARMAML
                4   FILE PHIC
              847   FILE PHIN
            10198   FILE PROMT
               56   FILE RDISCLOSURE
           105864   FILE SCISEARCH
                9   FILE SYNTHLINE
            49527   FILE TOXCENTER
            68363   FILE USPATFULL
             2252   FILE USPAT2
              112   FILE VETB
              741   FILE VETU
            27947   FILE WPIDS
            27947   FILE WPINDEX
L1               QUE YEAST
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     FILE 'CAPLUS, BIOSIS, SCISEARCH, MEDLINE, EMBASE, USPATFULL, LIFESCI,
     PASCAL, BIOTECHNO, TOXCENTER, ESBIOBASE, BIOTECHDS, AGRICOLA, CABA,
     WPIDS, FSTA, JICST-EPLUS, FROSTI, IFIPAT, PROMT, CANCERLIT, BIOBUSINESS,
     CEABA-VTB' ENTERED AT 10:36:24 ON 10 FEB 2004
L2          14099 S L1 AND (ASCORBIC ACID OR VITAMIN C)
L3             39 S L2 AND (KETOGULONIC ACID)
L4             38 DUP REM L3 (1 DUPLICATE REMOVED)
L5            100 S L1 AND KLG
L6              0 S L5 AND (ASCORBIC ACID ESTER)
L7             16 S L5 AND (ARABOASCORBIC ACID)
L8              8 DUP REM L7 (8 DUPLICATES REMOVED)
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=> d l4 ibib ab 30-38

L4 ANSWER 30 OF 38 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 1990-09530 BIOTECHDS
TITLE: Metabolic pathway for 2-keto-L-gulonic acid formation in Gluconobacter melanogenus IFO 3293; L-sorbose-dehydrogenase and L-sorbosone-dehydrogenase activity in **ketogulonic acid** production
AUTHOR: Hoshino T; Sugisawa T; Tazoe M; Shinjoh M; Fujiwara A
CORPORATE SOURCE: Nippon-Roche
LOCATION: Department of Applied Microbiology, Nippon Roche Research Center, 200 Kajiwara, Kamakura, Kanagawa 247, Japan.
SOURCE: Agric.Biol.Chem.; (1990) 54, 5, 1211-18
CODEN: ABCHA6
DOCUMENT TYPE: Journal
LANGUAGE: English
AB During the course of screening for **ketogulonic acid** producers using Gluconobacter melanogenus IFO 3293 as parent strain, various metabolic mutants besides **ketogulonic acid** producers were found. Among them, a **ketogulonic acid** non-producer (C20), L-idonate accumulator (99-23), and L-idonate non-producer (101-25) were found. G. melanogenus UV10 (parent) and 101-25 were grown at 30 deg with agitation at 300 rpm and aeration at 1.0 vvm in a 5 l fermentor containing medium No.3B-D with 70% sorbose, 0.75% **yeast** extract and 0.05% glycerol, while strains E9, C20, 99-23 were grown at 30 deg with agitation 500 rpm and aeration 0.75 vvm in a 3 l fermentor containing medium No.5 with 1.5% **yeast** extract, 8% L-sorbose and 0.05% glycerol. The cellular and enzyme activities of these mutants were measured. The L-sorbosone pathway for **ketogulonic acid** formation from L-sorbose was confirmed. L-sorbose-dehydrogenase (EC-1.1.99.12), which catalyzes the conversion of L-sorbose to L-sorbosone, occurred in the membrane fraction, while L-sorbosone-dehydrogenase occurred in the cytosol fraction. The latter enzyme required NAD or NADP. (3 ref)

L4 ANSWER 31 OF 38 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 1990-09529 BIOTECHDS
TITLE: Microbial production of 2-keto-L-gulonic acid from L-sorbose and D-sorbitol by Gluconobacter melanogenus; **ketogulonic acid** production in **yeast** extract or corn steep liquor culture medium; **ascorbic acid** precursor
AUTHOR: Sugisawa T; Hoshino T; Masuda S; Nomura S; Setoguchi Y;Tazoe M
CORPORATE SOURCE: Nippon-Roche
LOCATION: Department of Applied Microbiology, Nippon Roche Research Center, 200, Kajiwara, Kamakura, Kanagawa 247, Japan.
SOURCE: Agric.Biol.Chem.; (1990) 54, 5, 1201-09
CODEN: ABCHA6
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Strain SPO1, producing 13 g/l of **ketogulonic acid** (KGA), an **ascorbic acid** precursor, was isolated as a spontaneous mutant of Gluconobacter melanogenus IFO 3293. For the enhancement of KGA productivity, further strain improvement studies of the mutant were performed. Culture was performed at 30 deg and controlled pH, temperature and dissolved oxygen concentration in 3-l fermentors containing 2 l of either No.5 medium YE (1.5% **yeast** extract, 0.25% MgSO4.7H2O, 0.05% glycerol, 1.5% CaCO3, 0.15% antifoam and 8-10% L-sorbose or D-sorbitol) or No.5 medium CSL (the same as No.5 medium YE except that 2% corn steep liquor was used instead of 1.5% **yeast** extract). A mutant, U13, produced about 60 g/l KGA from 100 g/l

L-sorbose. Mutant Z84 produced about 60 g/l KGA from 100 g/l D-sorbitol. During the fermentation from L-sorbose and D-sorbitol, 5 to 10 g/l L-idonic acid was produced as a by-product. L-idonic acid was converted to KGA before the end of fermentation. D-sorbitol is cheaper than L-sorbose as a substrate and corn steep liquor is cheaper than **yeast** extract. Metabolic pathways for KGA production are presented. (21 ref)

L4 ANSWER 32 OF 38 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1988:588837 CAPLUS
DOCUMENT NUMBER: 109:188837
TITLE: Fermentative manufacture of 2-keto-L-gulonic acid from L-sorbose
INVENTOR(S): Imai, Hiroshi; Sakane, Takeshi; Nogami, Akio
PATENT ASSIGNEE(S): Takeda Chemical Industries, Ltd., Japan; Institute for Fermentation Research
SOURCE: Jpn. Kokai Tokkyo Koho, 7 pp.
CODEN: JKXXAF
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| JP 63112989 | A2 | 19880518 | JP 1987-133113 | 19870528 |
| JP 07095957 | B4 | 19951018 | | |
| US 4892823 | A | 19900109 | US 1987-57979 | 19870604 |
| US 4933289 | A | 19900612 | US 1989-413384 | 19890927 |
| JP 07250671 | A2 | 19951003 | JP 1995-48208 | 19950308 |
| JP 2574661 | B2 | 19970122 | | |
| PRIORITY APPLN. INFO.: | | | JP 1986-131121 | 19860605 |
| | | | US 1987-57979 | 19870604 |

AB The title acid (I), useful as synthetic intermediate for L-**ascorbic acid**, is manufactured from L-sorbose (II) by fermentation with Pseudomonas sorbosoxidans. Thus, P. sorbosoxidans 526-21 (IFO 14501, FERM P-8750) was shake-cultured in a medium containing glucose, polypeptone, dry **yeast**, and CaCO3 at 28% for 2 days, and then shake-cultured in a medium containing polypeptone, casamino acids, dry **yeast**, salts, thiamine.HCl, and II at 28° for 3 days to produce I in 34.0 mol% yield based on II.

L4 ANSWER 33 OF 38 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1987:457454 CAPLUS
DOCUMENT NUMBER: 107:57454
TITLE: Process for the manufacture of **ketogulonic acid**
INVENTOR(S): Fujiwara, Akiko; Sugisawa, Teruhide; Shinjoh, Masako; Setoguchi, Yutaka; Hoshino, Tatsuo
PATENT ASSIGNEE(S): Hoffmann-La Roche, F., und Co. A.-G., Switz.
SOURCE: Eur. Pat. Appl., 17 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| EP 213591 | A2 | 19870311 | EP 1986-111793 | 19860826 |
| EP 213591 | A3 | 19881005 | | |
| EP 213591 | B1 | 19920325 | | |
| R: AT, BE, CH, DE, FR, GB, IT, LI, NL | | | | |
| AT 74163 | E | 19920415 | AT 1986-111793 | 19860826 |

| | | | | |
|---|---|---|---|---|
| DK 8604087 | A | 19870301 | DK 1986-4087 | 19860827 |
| JP 62048389 | A2 | 19870303 | JP 1986-202600 | 19860828 |
| US 5541108 | A | 19960730 | US 1994-266998 | 19940628 |
| PRIORITY APPLN. INFO.: | | | GB 1985-21359 | 19850828 |
| | | | GB 1986-17888 | 19860722 |
| | | | US 1986-899586 | 19860825 |
| | | | EP 1986-111793 | 19860826 |
| | | | US 1990-517972 | 19900430 |
| | | | US 1993-16478 | 19930210 |
| | | | US 1994-183924 | 19940118 |

AB **Vitamin C** precursor 2-keto-L-gulonic acid (I) is manufactured from L-sorbose and/or D-sorbitol by cultivation of Gluconobacter oxydans having a high activity of L-sorbose dehydrogenase or by its cell free extract G. oxydans U-13 was cultivated in a medium containing L-sorbose 100, glycerol 0.5, **yeast** extract 15.0 g/L and salts at 30° on a rotary shaker for 4 days to yield 64.4 g of I. The cell free extract was also able to convert 100 mg of L-sorbose to 25 mg of I.

L4 ANSWER 34 OF 38 USPATFULL on STN

| | | | |
|---|---|---|---|
| ACCESSION NUMBER: | 80:23403 USPATFULL | | |
| TITLE: | Destruction by fermentation of 2-ketogluconate in the presence of 2-ketogulonate | | |
| INVENTOR(S): | Kita, Donald A., Essex, CT, United States<br>Gagne, John W., Norwich, CT, United States<br>Fenton, Dennis M., Gales Ferry, CT, United States | | |
| PATENT ASSIGNEE(S): | Pfizer Inc., New York, NY, United States (U.S. corporation) | | |
| | NUMBER | KIND | DATE |
| PATENT INFORMATION: | US 4202942 | | 19800513 |
| APPLICATION INFO.: | US 1978-926262 | | 19780720 (5) |
| DOCUMENT TYPE: | Utility | | |
| FILE SEGMENT: | Granted | | |
| PRIMARY EXAMINER: | Jones, Raymond N. | | |
| ASSISTANT EXAMINER: | Warden, Robert J. | | |
| NUMBER OF CLAIMS: | 2 | | |
| EXEMPLARY CLAIM: | 1 | | |
| LINE COUNT: | 195 | | |

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB 2-Ketogluconate present in a mixture with 2-ketogulonate is destroyed by fermentation with a strain of a Pseudomonas species leaving desired 2-ketogulonate intact. Subsequent hydrolysis of the 2-ketogulonate yields **ascorbic acid**.

L4 ANSWER 35 OF 38 CAPLUS COPYRIGHT 2004 ACS on STN

| | |
|---|---|
| ACCESSION NUMBER: | 1965:54890 CAPLUS |
| DOCUMENT NUMBER: | 62:54890 |
| ORIGINAL REFERENCE NO.: | 62:9742h,9743a |
| TITLE: | Manufacture of 2-keto-L-gulonic acid |
| PATENT ASSIGNEE(S): | Takeda Chemical Industries, Ltd. |
| SOURCE: | 29 pp. |
| DOCUMENT TYPE: | Patent |
| LANGUAGE: | Unavailable |
| FAMILY ACC. NUM. COUNT: | 1 |

PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| FR 1376741 | | 19641030 | FR | |
| GB 994119 | | | GB | |
| US 3234105 | | 1966 | US | |
| PRIORITY APPLN. INFO.: | | | JP | 19620920 |

AB 2-Keto-L-gulonic acid (I) was produced from sorbitol (II) by oxidation with

Aceto-bacter or Pseudomonas apparatus and the products converted to L-**ascorbic acid** (III) by enolization and lactonization. The bacteria were grown on media containing 2-5% II, pH 5-8, temperature 28-29°, with aeration, for about 150 hrs. Glycerol, glucose, or other carbohydrates could be added as supplementary C sources, and organic and inorg. N and minerals were required. A typical medium contained II 5%, glucose 0.5%, **yeast** extract 0.5%, and CaCO3 2.0%. Yield of I was 4.3 g./l. I could be recovered as such, or the broth (about 15 l.) could be decolorized, filtered, treated with Amberlite IR-120, type H, and dried in vacuo. The residue was dissolved in 700 ml. of MeOH, treated with activated charcoal, filtered, 0.9 ml. of concentrated H2SO4 added, and the solution again filtered. It was then heated for 3 hrs. with stirring, the MeOH removed by distillation, the residue washed with MeOH, and dried. L-**Ascorbic acid**, 22.5 g., was recovered. A similar procedure using Amberlite IRA-400, BuOH, HCl, and benzene could be employed.

L4 ANSWER 36 OF 38 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1954:72380 CAPLUS
DOCUMENT NUMBER: 48:72380
ORIGINAL REFERENCE NO.: 48:12885f-h
TITLE: Oxidative fermentation. XII. A new synthesis of **vitamin C**. 2
AUTHOR(S): Hori, Ichiro; Nakatani, Tsuneo
CORPORATE SOURCE: Osaka Univ.
SOURCE: Hakko Kogaku Zasshi (1954), 32, 33-6
CODEN: HKZAA2; ISSN: 0367-5963
DOCUMENT TYPE: Journal
LANGUAGE: Unavailable
AB cf. C.A. 48, 5275d. 2-**Ketogulonic acid** was determined by Fehling solution For the fermentation of Pseudomonas fluorescens, **yeast** water, corn-steep liquor, meat extract, and (NH4)2SO4 as sources of N gave equivalent results, but the N concentration greatly influenced fungus growth. A rapid increase of pH occurred in every concentration of (NH4)2SO4, reaching a maximum at the 7th day of fermentation; higher N concentration gave more vigorous N metabolism with consequent greater fungus growth and larger pH increase. The pH decreased markedly after the maximum, possibly due to H2SO4 liberation. With **yeast** water as a N source, pH increased more rapidly and decreased more slowly than with (NH4)2SO4. It was indicated that below pH 5.0 the fungus propagation was difficult and above pH 6.0 2-**ketogulonic acid** was accumulated to some extent; the optimum pH for the oxidation was 5.2-5.6. Addition of 0.2% KH2PO4 suppressed the increase of pH and gave good yields of 2-**ketogulonic acid**.

L4 ANSWER 37 OF 38 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1955:61137 CAPLUS
DOCUMENT NUMBER: 49:61137
ORIGINAL REFERENCE NO.: 49:11774a-c
TITLE: Preparation of the intermediate of **vitamin C** from the reduction products of 5-ketogluconate by oxidizing bacteria
AUTHOR(S): Kudaka, Masanobu; Aida, Hiroshi; Miyamoto, Ken
CORPORATE SOURCE: Tanabe Pharm. Co., Tokyo
SOURCE: J. Fermentation Assoc. (Hakko Kyokaishi) (1953), 11, 251-6
DOCUMENT TYPE: Journal
LANGUAGE: Unavailable
AB Soluble Ca gluconate (I), Ca L-idonate (II), and a reduction product (III) of 5-ketogluconate (IV) were fermented separately with Acetobacter suboxydans 621 and Gluconoacetobacter cerinus, nov. spec., and the change of these substrates was followed by determining Ca in 3 types, i.e., soluble, insol., and

insol. and reducing (equivalent to IV). The Acetobacter did not assimilate II, but assimilated I and produced about 70% IV. The Gluconoacetobacter acted both on I and II, and produced an insol. Ca salt. IV, produced from I, was also oxidized by the Gluconoacetobacter giving an insol. Ca salt. About 70% of I in III was converted to Ca-IV by the Acetobacter and II in the III was separated in solution, yield 95%, purity 90%. Four strains of Pseudomonas were selected for the activity of reducing idonic acid to 2-**ketogulonic acid** (V). Preparation of V from II by Pseudomonas fluorescens was improved by adding 1% **yeast** autolyzate, and the yield was 65%. When II obtained from III by the Acetobacter was used, the yield of V decreased about to 50% of that from pure II.

L4 ANSWER 38 OF 38 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1947:28286 CAPLUS
DOCUMENT NUMBER: 41:28286
ORIGINAL REFERENCE NO.: 41:5683h-i,5684a-d
TITLE: 2-**Ketogulonic acid** and its salts
INVENTOR(S): Gray, Byron E.
DOCUMENT TYPE: Patent
LANGUAGE: Unavailable
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| US 2421611 | | 19470603 | US | |

AB 2-**Ketogulonic acid** or its Ca salt valuable for the production of **vitamin C** are produced by oxidative fermentation of L-idonic acid or its Ca salt in the presence of Pseudomonas mildenbergii. The L-idonic acid is prepared along with D-gluconic acid by the hydrogenation of a soluble salt of 5-ketogluconic acid. The D-gluconic acid is transformed back to 5-ketogluconate by oxidative fermentation. Thus, a suspension of 172 parts of Ca 5-ketogluconate.2.5 H2O in 200 parts H2O is cooled to 20° and neutralized with H2SO4 below 20°. The mixture is then neutralized to litmus with NH3 or NH4OH, heated to 50°, and filtered. The filtrate is adjusted to pH 8.0-8.4, treated with 25 parts Raney Ni catalyst, and heated to 60° under 100 atmospheric H in an agitated autoclave until a sample no longer reduces Fehling's solution (4-6 hrs.). The catalyst is filtered. The filtrate is treated with 75 parts Ca(OH)2 and heated until all NH3 is driven off. The mixture is cooled, neutralized with CO2, treated with active C, filtered, and concentrated to 1000 parts. Glucose (75 parts), 6 parts corn steeping liquor, and 0.3 part octadecyl alc. are added, the pH is adjusted to 6 ± 0.1, and the mixture placed in a fermentation vessel. The solution is sterilized by heating 15 minutes at 15 lbs. steam pressure, cooled, treated with 17 parts sterile CaCO3, inoculated with 50 parts 48-hr. culture of Acetobacter suboxydans (grown on 5% sorbitol-0.5% **yeast** extract liquid tube culture) and agitated vigorously with sterile air for 8 days. The insol. Ca 5-ketogluconate (175 parts) is filtered and reused in the process. The filtrate containing Ca L-idonate is treated with 3 parts maltose (or glucose), 3 parts corn steeping liquor, 0.3 part KH2PO4 and 0.1 part MgSO4.7H2O, adjusted to pH 5.5-6.0, and sterilized 0.5 hr. at 15 lbs. steam pressure. The sterile mixture is inoculated with 50 parts 5% glucose-0.5% **yeast** liquid tube culture of Pseudomonas mildenbergii and vigorously agitated with sterile air for 10 days. The solution is clarified with active C, filtered, concentrated under reduced pressure and cooled. The concentrated solution is treated with 2 vols. EtOH, and the precipitate is filtered and dried to yield 45 parts Ca 2-keto-L-gulonate: The process is discussed in detail.

=> d l4 ibib ab 19-29

L4 ANSWER 19 OF 38 USPATFULL on STN
ACCESSION NUMBER: 2002:50802 USPATFULL
TITLE: Computer readable genomic sequence of Haemophilus influenzae Rd, fragments thereof, and uses thereof
INVENTOR(S): Fleischmann, Robert D., Gaithersburg, MD, United States
Adams, Mark D., N. Potomac, MD, United States
White, Owen, Gaithersburg, MD, United States
Smith, Hamilton O., Towson, MD, United States
Venter, J. Craig, Potomac, MD, United States
PATENT ASSIGNEE(S): Human Genome Sciences, Inc., Rockville, MD, United States (U.S. corporation)

| | NUMBER | KIND | DATE |
|---|---|---|---|
| PATENT INFORMATION: | US 6355450 | B1 | 20020312 |
| APPLICATION INFO.: | US 1995-476102 | | 19950607 (8) |

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 1995-426787, filed on 21 Apr 1995, now abandoned
DOCUMENT TYPE: Utility
FILE SEGMENT: GRANTED
PRIMARY EXAMINER: Campell, Bruce R.
NUMBER OF CLAIMS: 88
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 47 Drawing Figure(s); 47 Drawing Page(s)
LINE COUNT: 4666
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention provides the sequencing of the entire genome of Haemophilus influenzae Rd, SEQ ID NO: 1. The present invention further provides the sequence information stored on computer readable media, and computer-based systems and methods which facilitate its use. In addition to the entire genomic sequence, the present invention identifies over 1700 protein encoding fragments of the genome and identifies, by position relative to a unique Not I restriction endonuclease site, any regulatory elements which modulate the expression of the protein encoding fragments of the Haemophilus genome.

L4 ANSWER 20 OF 38 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2002-11486 BIOTECHDS
TITLE: Enhancing host cell's biosynthetic production of desired compound that is derived from partially intracellular pathway by increasing transport of extracellular substrate used in the synthetic pathway;
vector-mediated gene transfer and expression in Pantoea sp. or Klebsiella sp. and sorbosone-dehydrogenase
AUTHOR: KUMAR M; VALLE F
PATENT ASSIGNEE: GENENCOR INT INC
PATENT INFO: WO 2002012481 14 Feb 2002
APPLICATION INFO: WO 2000-US24600 4 Aug 2000
PRIORITY INFO: US 2000-677032 29 Sep 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-241757 [29]
AB DERWENT ABSTRACT:
NOVELTY - Enhancing biosynthesis by host cell (I) of compound (C) derived from an at least partially intracellular pathway (IP) of (I), comprising selecting (I) that has an IP using extracellular substrate (ES), increasing transport of ES into (I) while maintaining integrity of (I), where transport is rate limiting step in biosynthesis by (I), culturing (I) to produce ES, and producing (C), is new.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) enhancing (M1) transport of a substrate into the cytosol across the inner cell membrane involves selecting (I) and transforming

into (I) DNA encoding for one or more enzymes which transport the substrate into the host cell; and (2) overexpressing an anion/cation symporter involves selecting (I) and transforming into (I) DNA coding for one or more anion/cation symporters.

WIDER DISCLOSURE - Expression vectors and systems for enhanced production of desired end product by a host cell (preferably, microorganism), are also disclosed.

BIOTECHNOLOGY - Preferred Method: Increasing the transport of ES into (I) involves transforming into (I) DNA encoding for one or more polypeptides that increase the transport of the substrate into (I), the DNA preferably encodes one or more polypeptides which function as transport promoters e.g. major facilitator superfamily transporter, preferably anion/cation H+ symporter. In (M1), a host cell such as bacteria (e.g. Pantoea) or **yeast** is employed.

USE - Enhancing a host cell's biosynthetic production of a desired compound derived from an at least partially intracellular pathway in a host cell (claimed) preferably a microorganism such as Klebsiella and Pantoea. The method is particularly useful in conjunction with **ascorbic acid** intermediate synthesis, for example the conversion of 2,5-diketogluconic acid (2,5-DKG) to 2-keto-L-gulonic acid (2-KLG); the conversion of sorbose or sorbitol to 2-KLG via sorbosone; the reduction of 5-keto-D-Gluconic acid (5-KDG) to L-idonic acid; and the reduction of 5-keto-D-Gluconic acid to L-gulonic acid. Each of these pathways is characterized by a portion of the synthetic pathway, a synthetic reaction, that resides within the cytoplasm, e.g., the reduction of 2,5-DKG by 2,5 DKG reductase; the reduction of L-sorbosone to 2-KLG by a sorbosone dehydrogenase; the reduction of the 5-keto-D-gluconic acid (5-KDG) to L-idonic acid by 5-KDG dehydrogenase; and the reduction of 5-keto-D-gluconic acid to L-gluconic acid by 5-KDG reductase. These pathways were also characterized by the necessity of transporting the substrate, e.g., 2,5-DKG; L-sorbosone, etc., across the membrane for bioconversion by the synthetic reaction residing in the cytoplasm.

ADVANTAGE - Production of desired chemical compounds by recombinant microorganisms is increased by alleviating a limiting factor to a desired end product production.

EXAMPLE - Rate of bioconversion of 2,5-diketogluconic acid (2,5-DKG) to 2-keto-L-gulonic acid (2KLG) was determined to be dependent upon the import rate of 2,5-DKG into the cell. Thus increasing the DKG uptake transporters by overexpression can enhance the important rate of 2,5-DKG and will enhance 2KLG production rate. A 2,5-DKG transporter was discovered in Klebsiella oxytoca using DKG uptake assay. Previous works had shown that K. oxytoca comprised an operon designated yia operon, which contained eight putative open reading frames. Disruption of this operon removed the ability of K. oxytoca to use **ascorbic acid** as sole carbon source. One of the open reading frames in the yia operon, designated as yiaX2, encoded a transporter type transmembrane protein and was thus considered a candidate for 2,3-DKG permease. Since 2,3-DKG and 2,5-DKG were analogous molecules, it was possible that yiaX2 can transport 2,5-DKG and other sugar keto acids such as 2KLG. In order to determine if yiaX2 can transport 2,5-DKG, and 2-KLG this gene was deleted from the chromosome of K. oxytoca strain M5a1. The yiaX2 deletion mutant designated as MGK002 was created. Another K. oxytoca strain designated as Tester strain was created by adding back the plasmidly encoded and lac operon regulated yiaX2 gene to K. oxytoca strain MGK002. DKG uptake assay under +/- isopropylthiogalactoside (IPTG) induction using MGK002 and Tester strain confirmed that yiaX2 encoded a polypeptide having 2,5-DKG transport activities. A selection host (K. oxytoca with yiax2 gene) for finding 2,5-DKG transporter protein of Pantoea citrea and other biological sources was designed based on information that yiaX2 gene encoded transporter protein having 2,5-DKG transport activities. The resulting tester strain of K. oxytoca was yiaX2(tkr idno) and had all components needed for growth on 2,5-DKG as a sole carbon source except its inability to import DKG into the cytoplasm. Therefore, a nucleic acid

molecule that encodes a 2,5-DKG permease, upon expression in the tester strain, should confer the ability of the tester strain to grow on 2,5-DKG. Genomic DNA from P. citrea (ATCC 39140) was isolated using standard protocol and genomic library was created. The genomic library was introduced into tester strain K. oxytoca yiaX2(tkr idno) strain. Clones that grew on 2,5-DKG using M9-agar plates with 2.5 % 2,5-DKG and 0.1 mM IPTG were tested for DKG uptake using radiolabeled 14C (U) DKG. Various clones were found to have improved DKG uptake than the control tester strain. Genomic library DNA from these positive clones was transformed into P. citrea (139-2A) and DKG uptake assay was performed to measure the improvement in DKG uptake over the P. citrea 139-2A strain. Three to five fold improvement in DKG uptake rate was seen in the transformants having additional copies of plasmid encoded DKG permeases found through genomic library screening and selection methodology. Also the production rate of KLG production improved from 2.5 g/l to 3.2 g/l and yield on sugar improved from 45 % to 53 %.(60 pages)

L4 ANSWER 21 OF 38 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2002-09387 BIOTECHDS
TITLE: Biotechnological approaches for L-**ascorbic acid** production; involving **ketogulonic acid** conversion, fermentation, bioreactor and random mutagenesis for use in food, feedstuff, food-additive and cosmetic industry; a review
AUTHOR: HANCOCK RD; VIOLA R
CORPORATE SOURCE: Scottish Crop Res Inst
LOCATION: Hancock RD, Scottish Crop Res Inst, Unit Plant Biochem, Invergowrie, Dundee DD2 5DA, Scotland
SOURCE: TRENDS IN BIOTECHNOLOGY; (2002) 20, 7, 299-305
ISSN: 0167-7799
DOCUMENT TYPE: Journal
LANGUAGE: English
AB AUTHOR ABSTRACT - Over the past decade there has been increasing pressure to develop alternatives to the Reichstein process, a largely chemical synthesis by which the vast majority of world **vitamin C** (L-**ascorbic acid**, L-AA) is produced. The pressures include increasing environmental concerns and legislation, and theneed to increase process efficiency and reduce capital costs. The development of efficient fermentation processes in the past ten years has also represented a catalyst for change. Here, we describe the development of biotechnological alternatives for the synthesis of Reichstein intermediates by industrial microorganisms. The recent elucidation of the plant biosynthetic pathway represents new opportunities not only for the direct synthesis Of L-AAby fermentation but also for the production of human crop plants and animal fodder with enhanced nutritional value. We discuss the potential for these developments in the light of recent findings concerning L-AA biosynthesisin plants.
DERWENT ABSTRACT: L-**ascorbic acid** production is reviewed with respect to: bacterial biotransformations for the production of Reichstein intermediates (Gluconobacter oxydans gives almost 100% yields of L-sorbose from D-sorbitol, which is involved inproduction of L-**ascorbic acid**); production of L-**ascorbic acid** by mixed cultures; production of L-**ascorbic acid** by metabolic engineering to combine several enzyme activities within a single microorganism e.g. introduction of Corynebacterium sp.2,5-diketoglutarate-reductase gene into Erwinia herbicola to allow conversion of D-glc to 2-keto-L-gulonate or production of over 120 g/l 2-keto-L-gulonate in transgenic Pantoea citrea strains capable of converting D-glc directly to 2-keto-L-gulonate;continuous biocatalytic systems e.g. D-glc conversion to D-gluconate via NADP-dependent soluble glucose-dehydrogenase from Thermoplasma acidophilum; bioconversion of 2-keto-L-gulonate to L-**ascorbic acid** using hydrolase and lactonase; direct biosynthesisof L-

**ascorbic acid** e.g. in plants and **yeast**;
production of L-**ascorbic acid** in transgenic plants
(e.g. lettuce (Lactuca sativa)) and transformed alga e.g. Chlorella
pyrenoidosa; and direct L-**ascorbic acid** production in
**yeast**(7 pages)

L4 ANSWER 22 OF 38 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2002-11978 BIOTECHDS
TITLE: Novel bacterial strains belonging to genera Gluconobacter, Ketogulonogenium and Bacillus useful for producing 2-keto-L-gulonic acid from D-sorbitol via L-sorbose by fermentation;
vector-mediated gene transfer and expression in Gluconobacter sp., Ketogulonogenium sp. and Bacillus sp. for strain improvement
AUTHOR: LIAW H J; KOWZIC R L; EDDINGTON J M; YANG Y
PATENT ASSIGNEE: ARCHER-DANIELS MIDLAND CO
PATENT INFO: WO 2001083798 8 Nov 2001
APPLICATION INFO: WO 2000-US12037 4 May 2000
PRIORITY INFO: WO 2000-12037 4 May 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-240308 [29]
AB DERWENT ABSTRACT:
NOVELTY - A biologically pure culture (I) of microorganism strain comprising the identifying characteristics of a strain such as Ketogulonogenium robustum NRRL B-30265 (ADM 178-49) (M1), Gluconobacter oxydans NRRL B-30266 (ADM 205-95) (M2), Bacillus cereus NRRL B-30267 (ADM C12B) (M3), B.cereus NRRL B-30268 (ADM 1A9) (M4), or mutants derived from theses strains, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a microorganism culture system (II) comprising a mixture formed from a biologically pure culture of a microorganism strain having the identifying characteristics of (M2) and a biologically pure culture of a microorganism strain having the identifying characteristics of (M1), where the culture system is capable of producing at least about 40 g/l of 2-keto-L-gulonic acid (2-KLG) from D-sorbitol; and (2) transforming a strain by inserting a vector into the strain.

BIOTECHNOLOGY - Preferred Culture: (I) comprises a marker gene which comprises a nucleotide sequence which operatively directs synthesis of a protein conferring antibiotic resistance in a host cell. Preferably, the marker gene provides resistance to antibiotics such as ampicillin, chloramphenicol, erythromycin, kanamycin, spectinomycin, streptomycin or tetracycline. The vector further comprises an exogenous terminator of transcription, an exogenous promoter, and a discrete series of restriction endonuclease recognition sites, the series being between the promoter and the terminator.

USE - (I) having the identifying characteristics of (M1) or its mutant, is useful for producing 2-KLG which involves culturing (I) having the identifying characteristics of (M1) or its mutant, in mixed culture with a microorganism strain capable of converting D-sorbitol to L-sorbose in a medium containing D-sorbitol such that the D-sorbitol is converted to 2-KLG; and recovering the 2-KLG. The microorganism strain capable of converting a D-sorbitol to L-sorbose is preferably G.oxydans ATCC 621 or its mutant derived from the strain. The mutant derived from G.oxydans ATCC 621 is (M2) which is selected from media containing at least 100 g/l of L-sorbose. The microorganism having the identifying characteristics of (M1) preferably corresponds to (M1), and the microorganism strain capable of converting D-sorbitol to L-sorbitol is (M2). The mixed culture is capable of producing at least 40 g/l of 2-KLG from D-sorbitol. The 2-KLG is recovered as its salt from the medium and the recovered salt is converted to **ascorbic acid** or its salt. The microorganisms are cultured at a pH of about 5-9, and at a temperature of 5-36 degreesC. D-sorbitol is provided in the medium at a concentration of

20-250 g/l of medium. The inoculum ratio of (I) having identifying characteristics of (M1) to the L-sorbose producing strain is about 10:1 to 1:10. Preferably, the mixed culture comprises at least one additional microorganism strain of the genus Aureobacterium, Corynebacterium, Bacillus, Brevibacterium, Pseudomonas, Proteus, Enterobacter, Citrobacter, Erwinia, Xanthomonas and Flavobacterium, preferably B.cereus strain NRRL B-30267 or its mutant derived from the strain, where the mutant is selected to be incapable of producing the spores and is most preferably NRRL B-30268. The medium further comprises a soybean product such as soyflour, soyprotein and its hydrolysate, soy peptone, soluble soy isolates, soy whey or soy molasses. The products such as soluble soy isolates or soy whey are derived from the processing of soybeans (all claimed).

ADVANTAGE - The method involving (M1) and (M2) for producing 2-keto-L-gulonic acid (2-KLG) is simpler, having shorter fermentation with lower cost and higher yield for the production of 2-KLG from D-sorbitol in comparison with the conventional methods.

EXAMPLE - Mutagenesis and isolation of L-sorbose producing mutants from Gluconobacter oxydans ATCC 621 for single stage fermentation of 2-keto-L-gulonic acid (2-KLG), was carried out as follows. Bacterial cultures were grown to log phase in PYM medium (D-mannitol 50 g/l, glycerol 5 g/l, peptone 10 g/l, **yeast** extract 10 g/l, pH 7.0), then pelleted by centrifugation and resuspended in 2 ml of TM buffer Tris HCl 6.0 g/l, maleic acid 5.8 g/l, (NH4)2SO4 1.0 g/l, Ca(NO3)2 5.0 mg/l, MgSO4.7H2O 0.1 g/l, FeSO4.7H2O 0.25 mg/l, and autoclaved. The 2 ml cell suspension was mixed with 0.04 ml of 5.0 mg/ml solution of N'-nitro-nitrosoguanidine (NTG), then incubated at 30 degreesC for 30 minutes. After incubation, 10 ml of TM buffer was added to each tube, then the cells were pelleted, washed twice in TM buffer, then resuspended in 4.0 ml of 0.1 M NaH2PO4 buffer. The washed cell suspensions were further diluted in phosphate buffer, and aliquots were spread on plates of PYM medium, TBC medium. Difco Tryptone 5 g/l, **Yeast** extract 3 g/l, K2HPO4 7 g/l, KH2PO4 3 g/l, MgSO4 0.1 g/l, NaCl 5 g/l, D-sorbitol 10 g/l, tetrazolium blue chloride 0.03 g/l, agar 15 g/l, pH 6.8 or FM10 agar medium (corn steep liquor 2% (dry solid), ADM soy soluble 0.6% (dry solid), L-sorbose 150-200 g/l, CaCO3 50 g/l agar, 15 g/l, pH 7.2). These plates were incubated at 30 degreesC for 2-3 days. Colonies were then counted or isolated from these plates. Relative to unmutagenized control cells, the kill percentage from NTG treatment was 60-80%. Strain ADM 205-95 (NRRL B-30266), a mutant derived from ATCC 621 was isolated from FM10 agar medium. Subsequent tests of this strain were carried out in the shaker flask fermentation. One loopful culture of ADM 205-95 was inoculated into SM7 seed medium (Quest N-Z Soy 10 g/l, D-sorbitol 10 g/l, D-mannitol 20 g/l, corn steep liquor 2% dry solid, niacinamide 0.005 g/l, thiamine 0.3 g/l, pantothenic acid 0.4 g/l, p-aminobenzoic acid 0.2 g/l, pH 6.7), and incubated. Two ml of seed contents were used to inoculate 25 ml of fermentation medium FM11 (as described in FM10 medium except L-sorbose was replaced with 150-170 g/l D-sorbitol) in a 250 ml baffled shaker flask, and flasks were shaken for 24 hours at 30 degreesC and 240 rpm. The production of L-sorbose was assayed by high performance liquid chromatography (HPLC). Strain ADM 205-95 produced 145.8 and 153.7 g/l of L-sorbose with 91.6 and 93.4% of yield from D-sorbitol in these tests. Mutagenesis and isolation of 2-KLG producing mutants from Ketogulonogenium robustum ADM-X6L (NRRL B-21627) for the single stage fermentation of 2-KLG was carried out as follows. Bacterial cultures were grown in PYM medium to mid-log phase, then pelleted by centrifugation and resuspended in 2 ml of TM buffer. The 2 ml cell suspension was mixed with 60 microL of a 5.0 mg/ml solution of NTG, then incubated at 30 degreesC for 30 minutes. After incubation, 10 ml of TM buffer was added to each tube, then the cells were pelleted, washed twice in TM buffer. The cell suspension was further diluted in phosphate buffer, and aliquots were spread on plates of CM6 agar medium or CM6 medium containing 16-18% L-sorbose (CM6 medium contained Difco Bacto Soytone 10 g/l, D-sorbitol 5 g/l, D-mannitol 10 g/l, malt extract 5 g/l, **yeast** extract 5

g/l, K2HPO4 1 g/l, KH2PO4 9 g/l, NaCl 5 g/l, L-sorbose 160-180 g/l added separately after autoclaving). Plates were then incubated at 30 degreesC for 3 to 5 days. Colonies were counted from CM6 plates. Relative to control cells without NTG treatment, the killing percentage of NTG mutagenized cells was 32%. Colonies growing on CM6-18% L-sorbose plates were picked randomly and then screened for improved 2-KLG production from L-sorbose. One loopful culture of ADM 178-49 (NRRL B-30265) was inoculated into SM7 seed medium, and incubated at 30 degreesC and 240 rpm shaker for 22 hours. Two ml of seed contents were used to inoculate a baffled shaker flask containing 25 ml of fermentation medium FM10 and about 130 g/l of L-sorbose, and flasks were shaken for 72 hours at 30 degreesC and 240 rpm. Strain ADM 178-49 produced 62.6-67.8 g/l of 2-KLG with about 100% of yield from L-sorbose in these tests.(44 pages)

L4 ANSWER 23 OF 38 FROSTI COPYRIGHT 2004 LFRA on STN
ACCESSION NUMBER: 570280 FROSTI
TITLE: Bacterial strains and use thereof in fermentation process for 2-keto-L-gulonic acid production.
INVENTOR: Stoddard S.F.; Liaw H.J.; Eddington J.; Yang Y.
SOURCE: United States Patent
PATENT INFORMATION: US 6319699 B 20011120
APPLICATION INFORMATION: 19990413
NOTE: 20011120
DOCUMENT TYPE: Patent
LANGUAGE: English
AB This patent describes a method of producing 2-keto-L-gulonic acid (2-KLG), which is an intermediate step in the manufacture of **ascorbic acid**. It uses microorganisms that can ferment L-sorbose or D-sorbitol. Unlike previous methods, it does not need helper strains, **yeast** or expensive rare earth metals to produce 2-KLG efficiently.

L4 ANSWER 24 OF 38 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 1999-03755 BIOTECHDS
TITLE: Isolation and characterization of a new quinoprotein-dehydrogenase, L-sorbose/L-sorbosone-dehydrogenase; enzyme from Gluconobacter oxydans, used to produce the **ascorbic acid** intermediate 2-keto-L-gulonic acid
AUTHOR: Asakura A; Hoshino T
CORPORATE SOURCE: Nippon-Roche
LOCATION: Department of Applied Microbiology, Nippon Roche Research Center, 200 Kajiwara, Kamakura, Kanagawa 247-8530, Japan. Email: akira.asakura@roche.com
SOURCE: Biosci.Biotechnol.Biochem.; (1999) 63, 1, 46-53
CODEN: BBBIEJ
ISSN: 0916-8451
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Gluconobacter oxydans DSM 4025 is able to oxidize L-sorbose to 2-keto-L-gulonic acid (2KGA), which is a precursor of **ascorbic acid**. The enzyme that catalyzes the reaction was purified from the bacterium after culture in a medium containing L-sorbose, glycerol, urea, MgSO4.7H2O, corn steep liquor, baker's **yeast**, and CaCO3. It was characterized as a unique quinoprotein-dehydrogenase that catalyzes two reactions, the conversion of L-sorbose to L-sorbosone, and of L-sorbosone to 2KGA. The mol.weight of the enzyme was approximately 135,000, and consisted of two independent sub units, one of 64,500 and the other of 62,500. Non-covalently bound pyrroloquinoline quinone was found to be the prosthetic group. A dye-linked spectrophotometric enzyme assay revealed an optimum enzyme activity at a pH of 7-9, and that the enzyme was inhibited by EDTA and EGTA. The enzyme had a very wide substrate specificity, including primary and secondary alcohols, aldehydes, aldoses, ketoses, and other sugar alcohols, but not methanol

or formaldehyde. Cytochrome c derived from the soluble fraction of the bacterium proved to be a physiological electron acceptor of the enzyme. (12 ref)

L4 ANSWER 25 OF 38 FROSTI COPYRIGHT 2004 LFRA on STN
ACCESSION NUMBER: 482018 FROSTI
TITLE: Bacterial strains and use thereof in fermentation process for 2-keto-L-gulonic acid production.
INVENTOR: Stoddard S.F.; Liaw H.J.; Eddington J.; Yang Y.
PATENT ASSIGNEE: Archer Daniels Midland Co.
SOURCE: United States Patent
PATENT INFORMATION: US 5834231 B 19981110
APPLICATION INFORMATION: 19961024
NOTE: 19981110
DOCUMENT TYPE: Patent
LANGUAGE: English
AB This patent describes a method of producing 2-keto-L-gulonic acid (2-KLG), which is an intermediate step in the manufacture of **ascorbic acid**. It uses microorganisms that can ferment L-sorbose or D-sorbitol. Unlike previous methods, it does not need helper strains, **yeast** or expensive rare earth metals to produce 2-KLG efficiently.

L4 ANSWER 26 OF 38 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 1998-03994 BIOTECHDS
TITLE: Direct fermentation of 2-keto-L-gulonic acid in recombinant Gluconobacter oxydans; **ketogulonic acid** production
AUTHOR: Saito Y; Ishii Y; Hayashi H; Yoshikawa K; Noguchi Y; Yoshida S; Soeda S; Yoshida M
CORPORATE SOURCE: Fujisawa-Pharm.
LOCATION: Biotechnology Division, Pharmacological Research Laboratories, Fujisawa Pharmaceutical Co., Ltd., 2-1-6, Kashima, Yodogawa-ku, Osaka 532, Japan.
Email: yoshimasa_saito@fujisawa.rnd.co.jp
SOURCE: Biotechnol.Bioeng.; (1998) 58, 2-3, 309-15
CODEN: BIBIAU
ISSN: 0006-3592
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Gluconobacter oxydans T-100 (FERM BP-4188) and G624 (FERM BP-4415) were isolated and produced 2-keto-L-gulonic acid (KLGA) and L-sorbose, respectively. G. oxydans L-sorbose-dehydrogenase (EC-1.1.1.14) and L-sorbosone-dehydrogenase genes were cloned into plasmid pFG15A to form plasmid pSDH155. G. oxydans G624 was transformed with pSDH155 and cultured in a broth with 5% D-sorbitol, 0.5% **yeast** extract and 2.0% CaCO3 at 30 deg for 72 hr. Transformants produced 2.3-fold more 2-KLGA than G. oxydans T-100 whilst the parent strain produced none. Nitrosoguanidine mutagenesis was performed to create a low L-idonate-producing strain, IA1069; the plasmid was removed to leave the host strain G. oxydans NB 6939. When transformed with pSDH155, NB 6939 had a 190% higher KLGA yield than G624(pSHD155), resulting from a block in the L-idonate synthetic pathway. The T-100 promoter was replaced with Escherichia coli promoters, of which the tufB1 promoter in NB6939(pSDH155) resulted in a yield of 88 mg/ml 2-KLGA when cultured with 10% D-sorbitol. This method could be used for **ascorbic acid** production. (21 ref)

L4 ANSWER 29 OF 38 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 1990-09541 BIOTECHDS
TITLE: Production of 2-keto-L-gulonic acid from L-sorbose; **ketogulonic acid** preparation using strain DSM 4025 and Saccharomyces, Schizosaccharomyces, Pichia, Hansenula, Candida or Torulopsis cell or extract in culture medium
PATENT ASSIGNEE: Roche
PATENT INFO: EP 366922 9 May 1990
APPLICATION INFO: EP 1989-117499 21 Sep 1989
PRIORITY INFO: EP 1988-116156 30 Sep 1988
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 1990-140980 [19]
AB A process for production of 2-keto-L-gulonic acid, an **ascorbic acid** intermediate, is claimed, involving fermentation of L-sorbose by a mixture of bacterium DSM 4025 (which does not form **ketogulonic acid** when cultured alone) and a **yeast** or **yeast** product with growth promoting activity. The **yeast** is preferably an ascomycete, particularly Saccharomyces sp. (e.g. Saccharomyces cerevisiae (baker's **yeast**), Saccharomyces carlsbergensis (Saccharomyces uvarum) (brewer's **yeast**) or Saccharomyces sake), Schizosaccharomyces sp. (e.g. Schizosaccharomyces pombe), Pichia sp. (e.g. Pichia membranaefaciens) or Hansenula (e.g. Hansenula anomala), or a hyphomycete, particularly Candida sp. (e.g. Candida tropicalis or Candida utilis) or Torulopsis (e.g. Torulopsis versatilis (Candida versatilis) or Torulopsis holmii (Candida holmii)), added as a culture medium component, preferably after sterilization. The **yeast** is used at a concentration of 20-250 g/l (preferably 50-200 g/l). **Ketogulonic acid** is produced in a yield of at least 72 g/l, preferably at least 100 g/l, at pH 4.0-9.0 (preferably 6.0-8.0) and at 13-36 deg (preferably 18-33 deg). (11pp)

L5 ANSWER 4 OF 5 USPATFULL on STN
ACCESSION NUMBER: 2002:22144 USPATFULL
TITLE: VITAMIN C PRODUCTION IN MICROORGANISMS AND PLANTS
INVENTOR(S): BERRY, ALAN, BLOOMFIELD, NJ, UNITED STATES
RUNNING, JEFFREY A., MANITOWOC, WI, UNITED STATES
SEVERSON, DAVID K., TWO RIVERS, WI, UNITED STATES
BURLINGGAME, RICHARD P., MANITOWOC, WI, UNITED STATES

| | NUMBER | KIND | DATE | |
|---|---|---|---|---|
| PATENT INFORMATION: | US 2002012979 | A1 | 20020131 | |
| APPLICATION INFO.: | US 1999-318271 | A1 | 19990525 | (9) |

| | NUMBER | DATE | |
|---|---|---|---|
| PRIORITY INFORMATION: | US 1998-88549P | 19980608 | (60) |
| | US 1999-125073P | 19990317 | (60) |
| | US 1999-125054P | 19990318 | (60) |

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: SHERIDAN ROSS PC, 1560 BROADWAY, SUITE 1200, DENVER, CO, 80202
NUMBER OF CLAIMS: 72
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 12 Drawing Page(s)
LINE COUNT: 8483
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A biosynthetic method for producing vitamin C (ascorbic acid, L-ascorbic acid, or AA) is disclosed. Such a method includes fermentation of a genetically modified microorganism or plant to produce L-ascorbic acid. In particular, the present invention relates to the use of microorganisms and plants having at least one genetic modification to increase the action of an enzyme involved in the ascorbic acid biosynthetic pathway. Included is the use of nucleotide sequences encoding **epimerases**, including the endogenous GDP-D-mannose:GDP-L-galactose **epimerase** from the L-ascorbic acid pathway and homologues thereof for the purposes of improving the biosynthetic production of ascorbic acid. The present invention also relates to genetically modified microorganisms, such as strains of microalgae, bacteria and yeast useful for producing L-ascorbic acid, and to genetically modified plants, useful for producing consumable plant food products.

L8 ANSWER 6 OF 8 USPATFULL on STN DUPLICATE 4

| | |
|---|---|
| ACCESSION NUMBER: | 2002:57576 USPATFULL |
| TITLE: | Production of ascorbic acid |
| INVENTOR(S): | Kumar, Manoj, Fremont, CA, United States |
| PATENT ASSIGNEE(S): | Genencor International, Inc., Rochester, NY, United States (U.S. corporation) |

| | NUMBER | KIND | DATE | |
|---|---|---|---|---|
| PATENT INFORMATION: | US 6358715 | B1 | 20020319 | |
| APPLICATION INFO.: | US 1998-205874 | | 19981204 | (9) |
| DOCUMENT TYPE: | Utility | | | |
| FILE SEGMENT: | GRANTED | | | |
| PRIMARY EXAMINER: | Prouty, Rebecca E. | | | |
| ASSISTANT EXAMINER: | Rao, Manjunath | | | |
| LEGAL REPRESENTATIVE: | Ito, Richard T. | | | |
| NUMBER OF CLAIMS: | 19 | | | |
| EXEMPLARY CLAIM: | 1 | | | |
| NUMBER OF DRAWINGS: | 3 Drawing Figure(s); 2 Drawing Page(s) | | | |
| LINE COUNT: | 812 | | | |

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention provides for the production of ASA from **yeast** capable of producing ASA from **KLG**. The present invention provides methods for the production of ASA as well as recombinant **yeast** capable of producing ASA from a carbon source.